52) Lipoprotein and Apolipoprotein in SHRSP---Isolation, Characterization and Age-related Change of Serum Apolipoprotein A-IV in SHRSP. Hiroshi Ogawa, Chiyo Shiota, Tomoyo Nishikawa and Sukenari Sasagawa. Department of Hygiene, Kinki University School of Medicine, Osaka-fu 589.

In our previous paper, we reported on characterization, concentration and distribution of serum apolipoprotein A-I(apo A-I) and apolipoprotein E(apo E) in SHRSP(Jpn. Heart J.,26:673-674,1985). In addition to apo A-I and apo E, in the rats, apolipoprotein A-IV(apo A-IV) is also major protein component of high density lipoprotein(HDL) which is well known to be an anti-atherogenic factor. To carry out the studies on lipoprotein and apolipoprotein metabolism in SHRSP, it is indispensable to investigate possible metabolic relationship among these apolipoproteins in SHRSP. In the present paper, we reported on 1)isolation and characterization of serum apo A-IV, 2)age-related change in serum apo A-IV concentration and 3)alteration of serum apo A-IV concentration and distribution by high-fat and -cholesterol(HFC) feeding.

MATERIALS AND METHODS: As a starting material for isolation of apo A-IV, the unbound fraction to Heparin-Sepharose affinity chromatography described in the isolation of apo A-I was used. Apo A-IV was finally isolated by FPLC system(Superose 12 HR10/30, Pharmacia) after Sephacryl S-200(Pharmacia) gel-filtration of the above unbound fraction. The purity of isolated apo A-IV was checked by SDS-PAGE and isoforms of apo A-IV were examined by isoelectric focusing(IEF) between pH 4.0-6.5. Densitometric scanning of isoforms pattern on stained IEF plate was performed by laser densitometer(LKB 2202 ULTROSAN). Monospecific antibody against apo A-IV was raised in male rabbits and electro-immunoassay(EIA) procedure for quantitation of apo A-IV was established by using antiserum to apo A-IV as well as apo A-I and apo E. Male SHRSP and age-matched male WKY at various ages until 12 months of age were used for study on age-related change of serum apo A-IV concentration. Blood was obtained from jugular vein under ether anesthesia after fasting overnight. Protocols for serum lipoprotein fractionation, feeding of HFC diet to the rats were the same as mentioned previously(Jpn. Heart J.,26:674,1985).

RESULTS AND DISCUSSION: Apo A-IV isolated from serum showed at least five isoforms(pI :5.10, 5.14, 5.23, 5.29 and 5.34 from anode) in IEF and ratios(%) of the isoforms were 3.1, 17.7, 36.7, 37.9 and 4.6 from anode. But, no significant difference of isoforms was observed between WKY and SHRSP. Antibody against apo A-IV didn't react with apo A-I, apo E from rat serum or diluted human serum, but gave single precipitant arc with isolated apo A-IV, diluted rat serum, HDL from rat or lipoprotein-free fraction from rat. Age-related change in the concentration of serum apo A-IV in both strains was almost constant throughout experimental period except 3 weeks of age without fasting, and the distribution between HDL and lipoprotein-free fraction didn't change with aging. These results suggest that neither serum concentration nor distribution of apo A-IV are influenced by aging in both strains. But, the concentrations in serum and HDL of SHRSP were significantly lower than those of WKY during the experimental period($p<0.05$), which suggests that serum apo A-IV concentration might be prominently affected by genetic factor. By the feeding of HFC diet to 16 week-old rats, apo A-IV concentration in both HDL and lipoprotein-free fraction of WKY slightly increased, resulting in significant increase of serum total apo A-IV. On the other hand, apo A-IV concentration in HDL of SHRSP was reduced significantly, while apo A-IV concentration in lipoprotein-free fraction was elevated significantly. Consequently, serum total apo A-IV concentration in SHRSP remained unchanged. These changes in the concentration of apo A-IV in HDL of both strains were relatively similar to those of apo A-I in HDL of both strains as reported previously, suggesting that there may be close relationship between apo A-I and apo A-IV in HDL of both strains. But, further studies will be necessary to define the increase of apo A-IV concentration in lipoprotein-free fraction of both strains.